EXPERIMENTS ON LIGIA IN BERMUDA

VI. Reactions to Common Cations

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Five preceding papers of this series (Barnes, 1932, 1934, 1935, 1936, 1938) have described the effect of salts and other factors on the littoral isopod *Ligia baudiniana*. The present report deals with the negative reaction of *Ligia* to filter paper moistened with salt solutions and the survival in sea water containing a higher percentage of K, Ca, or Na.

Reaction to Filter Paper Moistened with Salt Solutions

Large filter papers (diameter 25 cm.) were cut in two, each half saturated with a different solution and placed in a covered flat dish in a photographic dark room having a light of 61.9 foot candles directly above the center of the dish. Five isopods (freshly collected) were released in the dish and their distribution on the two halves was observed at five to fifteen-minute intervals for a period of one to two hours. The dish was rotated 90° after each observation to eliminate any unsuspected source of orientation. With distilled water on both sides the distribution was approximately equal.

It is known (Barnes, 1938) that the isopods tend to collect on the distilled water side when the other half of the paper is moistened with sea water and the present experiments were designed to test the salts separately. The aversion for sea water is shown by specimens previously kept in air on seaweed moistened with sea water (Barnes, 1938) and by specimens washed rapidly in distilled water (Barnes, 1935), but it was found that prolonged immersion in distilled water destroys this reaction. Thus in the present experiments of 168 specimens previously immersed separately in 100 cc. of distilled water for an average of half an hour, 82 collected on the sea water side and 86 on the distilled water paper.

For $\frac{5}{8}$ M NaCl *vs.* distilled water, the ratio is the same as for sea water *vs.* distilled water, and in both cases the negative reaction no longer occurs at 50 per cent dilution (Table I). The aversion for KCl paper was still evident in a dilution of 10 per cent (see Table I). The isopods showed a positive reaction to $CaCl_2$ paper compared with distilled water paper and an "all-or-none" reaction to $CaCl_2$ occurred when the other half contained NaCl. An even distribution occurred between $CaCl_2$ and LiCl paper and the negative reaction to LiCl compared with distilled water was less than that to the other salts.

Survival in Modified Sea Water

The toxic action of Na, Ca and K was studied by increasing the proportion of each salt in sea water. Specimens were tested separately in 100 cc. of mixtures of sea water and isotonic salt solution (Table II).

TABLE I

Reaction of Ligia to filter paper saturated with salt solutions.
(The animals were tested in groups of five.)

Treatment of each half of paper	Total number of isopods on each half	Ratio
5/8 M NaCl vs. distilled water	71 : 127	1 : 1.78
75 per cent 5/8 M NaCl vs. distilled water	85 : 120	1 : 1.41
50 per cent 5/8 M NaCl vs. distilled water	73 : 77	1 : 1.05
25 per cent 5/8 M NaCl vs. distilled water	83 : 72	1 : 0.86
5/8 M NaCl vs. $\frac{3.5}{8}$ M $CaCl_2$	1 : 63	1 : 63.00
100 per cent 5/8 M KCl vs. distilled water	37 : 147	1 : 3.97
75 per cent 5/8 M KCl vs. distilled water	26 : 82	1 : 3.15
50 per cent 5/8 M KCl vs. distilled water	31 : 81	1 : 2.61
25 per cent 5/8 M KCl vs. distilled water	56 : 82	1 : 1.46
10 per cent 5/8 M KCl vs. distilled water	61 : 99	1 : 1.62
5 per cent 5/8 M KCl vs. distilled water	96 : 104	1 : 1.08
$\frac{3.5}{8}$ M $CaCl_2$ vs. distilled water	105 : 55	1 : 0.52
$\frac{3.5}{8}$ M $CaCl_2$ vs. 5/8 M LiCl	22 : 22	1 : 1.00
5/8 M LiCl vs. distilled water	49 : 63	1 : 1.28

The isopods showed a high tolerance for sea water containing excessive sodium or calcium, but increasing the KCl content is rapidly fatal. The toxicity of Na is a smooth function of the concentration. The Ca curve is erratic and the K curve falls abruptly for sea water mixtures containing over $\frac{1}{16}$ M KCl (see Fig. 2).

Survival in Oxygenated Solutions

The wide variation in survival times in a given salt solution suggests

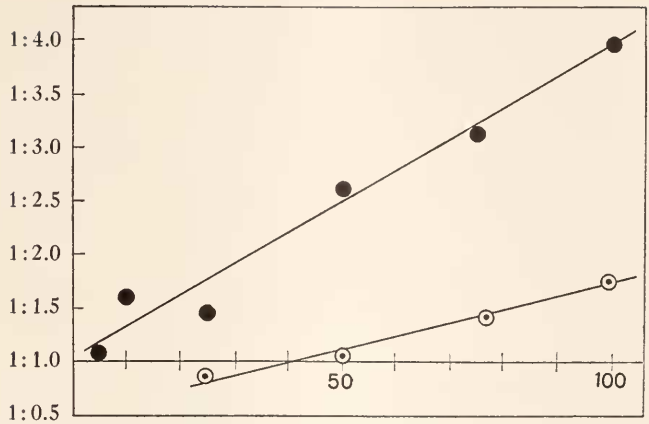

FIG. 1. Negative reaction to filter paper containing salt solutions. Ordinates: ratio of the number of isopods on the salt solution side to the number on the distilled water side. Abscissae: percentage concentration in cc. of 5/8 M salt. Solid circles: KCl. Open circles: NaCl (see Table I).

that other factors besides the direct chemical effect of the ions may be involved. To eliminate effects of asphyxiation oxygen was bubbled

TABLE II

Survival of Ligia in modified sea water

Parts of salt solution in 100 cc. of modified sea water	Average survival	Maximum survival	Number of specimens
	hours	*hours*	
9 per cent 5/8 M NaCl	69.8±15.2	136	9
50 per cent 5/8 M NaCl	59.4±4.5	168	39
71.4 per cent 5/8 M NaCl	30.9±3.5	79	20
80 per cent 5/8 M NaCl	20.7±0.8	24	37
90 per cent 5/8 M NaCl	8.0±0.03	11	43
10 per cent 5/8 M KCl	44.7±5.6	112	19
11 per cent 5/8 M KCl	36.5±5.5	48	8
20 per cent 5/8 M KCl	10.2±0.7	17	19
30 per cent 5/8 M KCl	3.7±0.4	6	8
40 per cent 5/8 M KCl	5±0	5	5
50 per cent 5/8 M KCl	0.7±.08	1	10
30 per cent $\frac{3.5}{8}$ M $CaCl_2$	68.5±10.8	264	25
50 per cent $\frac{3.5}{8}$ M $CaCl_2$	34.2±5.7	242	24
70 per cent $\frac{3.5}{8}$ M $CaCl_2$	39.1±6.7	254	19
90 per cent $\frac{3.5}{8}$ M $CaCl_2$	38.8±2.9	62	17

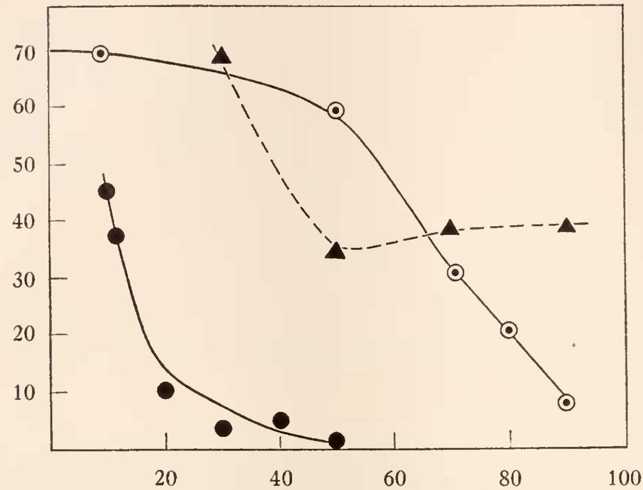

FIG. 2. Survival times of isopods in sea water in which the concentration of one ion is increased. Ordinates: average survival in hours. Abscissae: concentration of 5/8 M KCl and NaCl and of 3.5/8 M $CaCl_2$ in cc. per 100 cc. of modified sea water. Solid circles: KCl; open circles: NaCl; triangles: $CaCl_2$.

through the 100 cc. of salt solution in several cases, but did not affect the survival times in distilled water, 5/8 M NaCl or 5/8 M KCl (Table III).

TABLE III

Survival in oxygenated solutions

Medium	Average survival	Maximum survival	Number of specimens
	hours	*hours*	
Distilled water	4.0±0.2	6	8
5/8 M NaCl	8.7±0.6	11	4
5/8 M KCl	1.6±0.1	3	7

Discussion

It is of interest to note that the aversion for sea water is similar to aversion for NaCl and in both cases the negative reaction ceases at half dilution. The quantity of KCl in sea water is below the threshold for the negative reaction to this salt, while $CaCl_2$ has no repellent action when compared with distilled water.

It was suggested (Barnes, 1938) that the salts on the paper stimulate the isopods to greater movement which compels them to collect on the salt-free side. The pronounced aversion for KCl and positive reaction to Ca support this hypothesis. The rather precise relation between the concentration of NaCl and KCl and the magnitude of the response may also be cited. It is unfortunate that our quantitative knowledge of chemical stimulation is very meager compared with the extensive studies of stimulation by light and gravity. The ratio of effect of K *vs.* distilled water to Na *vs.* distilled water is 2.2, somewhat greater than the ratio of ionic mobilities, i.e., 1.5. Hopkins (1932) has shown the importance of ionic mobility in stimulation by salts. The reaction of the animals when each side of the substratum is treated with a different salt is not an additive effect of the reactions to each salt compared with distilled water paper. Thus the ratio for $CaCl_2$ *vs.* distilled water is 1 : 0.52 and NaCl *vs.* water is 1 : 1.78 but the ratio for $CaCl_2$ *vs.* NaCl is as high as 63.0 : 1. This striking difference probably results from the combined action of the inhibiting Ca and stimulating Na on the leg movements. The differences cannot be explained by toxicity alone. Thus Li, the most toxic of the ions, gives a 1 : 1 ratio with Ca.

In the case of sea water *vs.* distilled water the salt requirements of the animals must also be considered. Thus if depleted of salt by a half hour's immersion in distilled water the negative reaction to sea water disappears. The rôle of the flushing mechanism for the gills by which water rises between the last pair of legs is not known. Several animals were observed with the legs in position for the capillary conduit, but there was not sufficient solution on the filter paper to flush the gills.

The specific effect of Na, Ca, and K is indicated in the toxicity curves for sea water containing increasing concentrations of each ion. The smooth relation between concentration and toxicity for Na suggests a gradual modification of a normal process in the animal. The very steep KCl curve indicates severe toxic action and the lack of correlation with concentration in the case of calcium is characteristic of a surface reaction. It was shown (Barnes, 1938) that the protective action of Ca in hypotonic sea water also resembles a surface reaction.

The experiments in which oxygen was introduced into the solutions help to show that the reported survivals of immersed animals are true salt effects. This is especially important in the case of K which paralyzes all gill movement. It is probable that oxygen would increase the survival in less toxic solutions in which the animals live for several days.

Summary

1. The negative reaction of *Ligia* to filter paper moistened with 5/8 M NaCl is similar to the negative reaction to sea water. In both cases dilution by 50 per cent destroys the effect.

2. The aversion for filter paper containing KCl and NaCl is approximately a rectilinear function of the concentration.

3. The results can be explained by the assumption that the ions stimulate the legs and the greater activity results in a distribution on the salt-free side of the substratum.

4. When presented with a " choice " between filter paper containing $CaCl_2$ and NaCl, there is an " all-or-none " aversion for the NaCl.

5. The survival of *Ligia* in sea water containing increasing concentrations of Na, K and Ca is described.

6. The survival in distilled water or in solutions of single salts is not affected by oxygenation.

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